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RAPID COMMUNICATION

Enteropathy-Associated T Cell Lymphoma (Malignant Histiocytosis of the Intestine) Is Recognized by a Monoclonal Antibody (HML-1) that Defines a Membrane Molecule on Human Mucosal Lymphocytes

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Enteropathy-associated T cell lymphoma (EATCL; malignant histiocytosis of the intestine) arises in patients with enteropathy, which in some cases is known to be a result of gluten sensitivity. The lymphoma arises in the intestine, where it may remain localized, although eventual dissemination is the rule. Intraepithelial tumour cells often are seen at the mucosal tumor margin. These features suggest that EATCL may be a tumor of intraepithelial lymphocytes. A monoclonal antibody (HML-1) has been produced recently that recognizes the entire intraepithelial lymphocyte population and 50% of lamina propria T cells but very few cells outside the mucosa. Immunocytochemistry has shown that all cases of enteropathy-associated T cell lymphoma studied are HML-1 positive and all peripheral T cell lymphomas and mucosal B cell lymphomas are HML-1 negative. This suggests strongly that EATCL is a tumor of mucosal T cells, possibly the intraepithelial T cell component. (Am J Pathol 1988, 132:1–5)

IN 1978 ISAACSON AND WRIGHT characterised the small intestinal lymphoma that may complicate coeliac disease, describing it as a form of malignant histiocytosis.[1] Later studies, however, showed unequivocally that the tumor cells exhibited a T cell phenotype. This was confirmed by gene rearrangement studies, which showed clonal rearrangements of the genes encoding for the β-chain of the T cell receptor in 3 of 4 cases studied.[2,3] O'Farelly et al[4] questioned whether the villous atrophy seen in association with this form of lymphoma represented true coeliac disease. They found that, in contrast to true coeliacs, their patients with this type of lymphoma lacked antibodies to α-gliadin. Therefore, they coined the term enteropathy-associated T cell lymphoma (EATCL) for this tumor.

There is mounting evidence to suggest that EATCL arises from a mucosa-committed T cell. The disease starts in the intestine, where it may remain localized for long periods, although eventual wide dissemination is the rule. Simple resection of the involved intestine sometimes results in long remissions or even cure. Intraepithelial (IE) T cells are sometimes dramatically increased in the uninvolved mucosa in EATCL and the tumor cells themselves often are seen in the epithelium.[2,5] These observations suggest that EATCL may be a tumor of IE T cells.[6]

The monoclonal antibody HML-1, raised against IE T cells, recognizes all subsets of these cells and ap-

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Table 1—Antibodies Used in this Study

Antibody	Specificity	Reference
UCHT1	CD3	10
Leu3a	CD4	11
3A1	CD7	12
UCHT4	CD8	13
TO15	CD22	14
NKH1		Coulter clone
HML1	Mucosal T cells	7

proximately 50% of lamina propria T cells. HML-1 reacts with only a few T cells in other lymphoid compartments.[7] Reactivity of HML-1 with EATCL would provide strong supportive evidence for its origin from mucosal T cells, especially if other peripheral T cell lymphomas were negative. Accordingly, the authors have performed immunocytochemical staining of 8 cases of EATCL using HML-1 and compared the results with those obtained in cases of extraintestinal T and B cell lymphomas and gastrointestinal B cell lymphomas, which often have an intraepithelial component.

Materials and Methods

Tissue

Blocks of tumor from 8 cases of EATCL were snap frozen in liquid nitrogen and stored at −70 C. The details of these cases have been reported previously.[2,3] Snap frozen lymph nodes from 20 cases of extraintestinal peripheral T cell lymphoma were retrieved from storage at −70 C. These cases had been characterised in detail previously and classified according to the classification of Stansfeld.[8] They were comprised of cutaneous, anaplastic, large-cell pleomorphic, large-cell monomorphic, angio-immunoblasticlike, and immunoblastic T cell lymphomas. Also retrieved were frozen blocks from 24 cases of gastrointestinal B cell lymphomas. These included 10 cases of primary B cell gastric lymphoma that had been described as part of a previous series and had shown characteristic lymphoepithelial lesions.[9] Fifteen extraintestinal B cell lymphomas also were studied.

Immunohistochemistry

The source and specificity of the antibodies used are shown in Table 1.

Cryostat sections were cut at 8 μm, air dried, and fixed in acetone for 30 minutes. Staining was performed using the indirect immunoperoxidase technique, with rabbit anti-mouse immunoglobulin conjugated to peroxidase as the secondary antibody.[15] Reactivity was visualized using the diamino benzidene reagent.

Results

The results of immunohistochemical studies of tumor tissue are summarized in Table 2.

EATCL

With the exception of CD3 expression, all 8 cases exhibited the same phenotype, namely CD3±, CD7+, CD5−, CD4−, CD8−, HML-1+ (Figures 1 and 2). In 1 case the large bizarre tumor cells that were CD3+, HML-1+ could be distinguished clearly from accompanying reactive T cells that were CD3+, HML-1−. All cases of EATCL were NKH-1−.

Peripheral T Cell Lymphomas

The phenotype of these cases was highly variable but all except 1 expressed CD3. This case was CD7+, CD4−, CD8− and thus showed the same phenotype as EATCL, with the exception of HML-1 reactivity. Tumor cells in all other cases of peripheral T cell lymphoma were similarly HML-1−.

Gastrointestinal B Cell Lymphomas

These contained a moderately heavy reactive CD3+ T cell infiltrate with isolated intraepithelial T cells. Widely scattered HML-1 positive cells were present, some of which occurred as isolated intraepithelial cells in numbers comparable to the intraepithelial T cells. Well-defined lymphoepithelial lesions

Table 2—Phenotype of Lymphomas Studied

Tumor	Number tested	No. of positive cases						
		CD3	CD7	CD4	CD8	NKH1	CD22	HML-1
EATCL	8	5	8	–	–	–	–	8
Gastrointestinal B cell lymphomas	24	–	–	–	–	–	24	–
Peripheral T cell lymphomas	20	19	9*	14	8	NT	–	–
Peripheral B cell	15	–	–	–	–	–	15	–

* Of 9 tested.
NT, not tested.

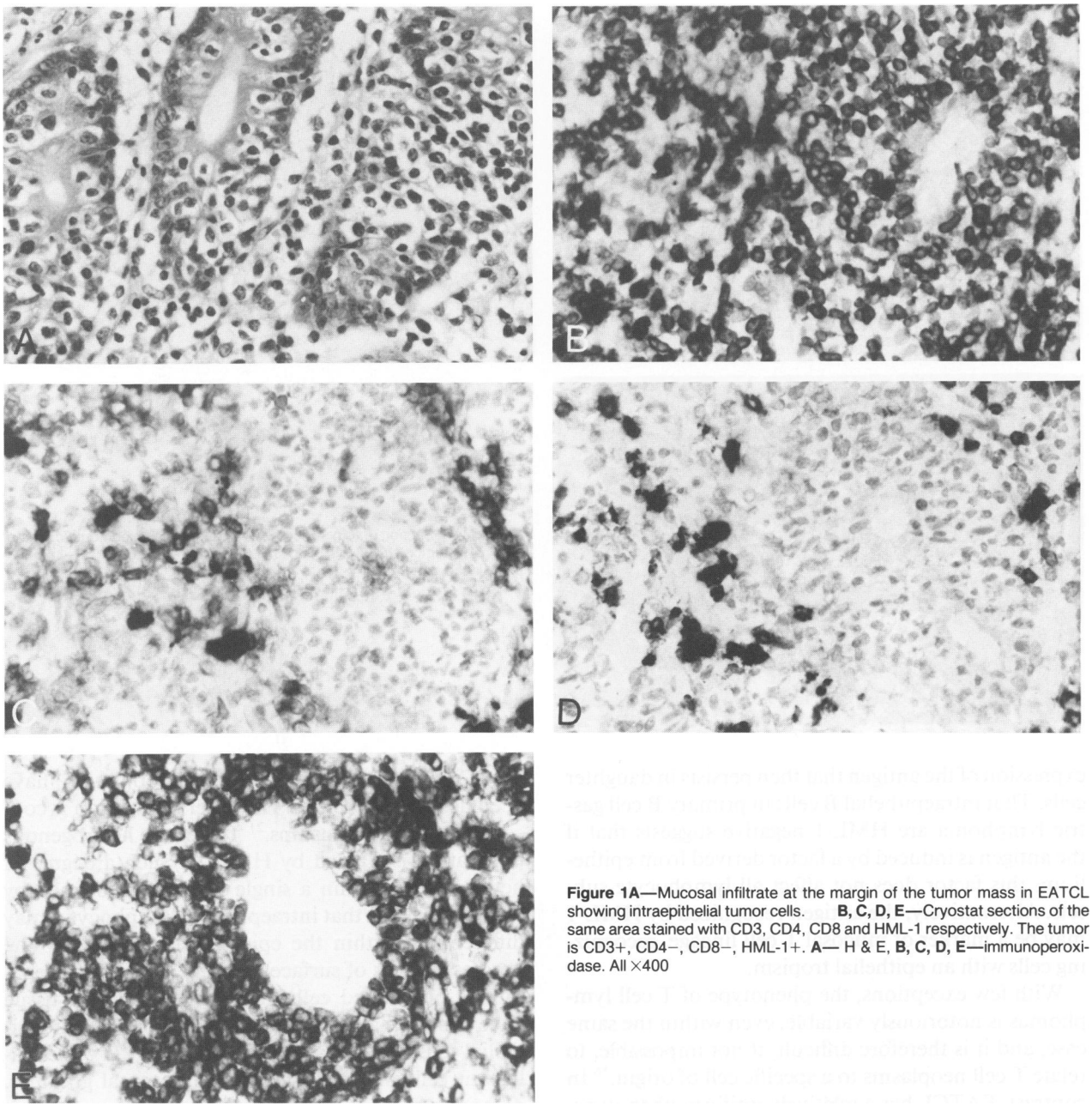

Figure 1A—Mucosal infiltrate at the margin of the tumor mass in EATCL showing intraepithelial tumor cells. **B, C, D, E**—Cryostat sections of the same area stained with CD3, CD4, CD8 and HML-1 respectively. The tumor is CD3+, CD4−, CD8−, HML-1+. **A**— H & E, **B, C, D, E**—immunoperoxidase. All ×400

were formed by the CD22+ tumor cells that were, however, HML-1−.

Extraintestinal B Cell Lymphomas

All extraintestinal B cell lymphomas together with infiltrating reactive T cells were HML-1 negative.

Discussion

This study shows that EATCL can be distinguished from other types of T cell lymphoma by its reactivity with the monoclonal antibody HML-1. This antibody recognizes predominantly mucosal T cells including the heterogenous IE lymphocyte population.[7] The nature of the antigen recognized by HML-1 and the significance of its expression are not yet known. Although some tumor cells in EATCL may be present within the epithelium, the tumor is highly invasive and characteristically spreads through all intestinal layers and to mesenteric nodes. HML-1 is strongly expressed by all the tumor cells. This suggests that this antigen is not adsorbed passively from epithelial cells, but is actively synthesized by intestinal T lymphocytes. It is conceivable however that it is the initial association with mucosal epithelium that induces the

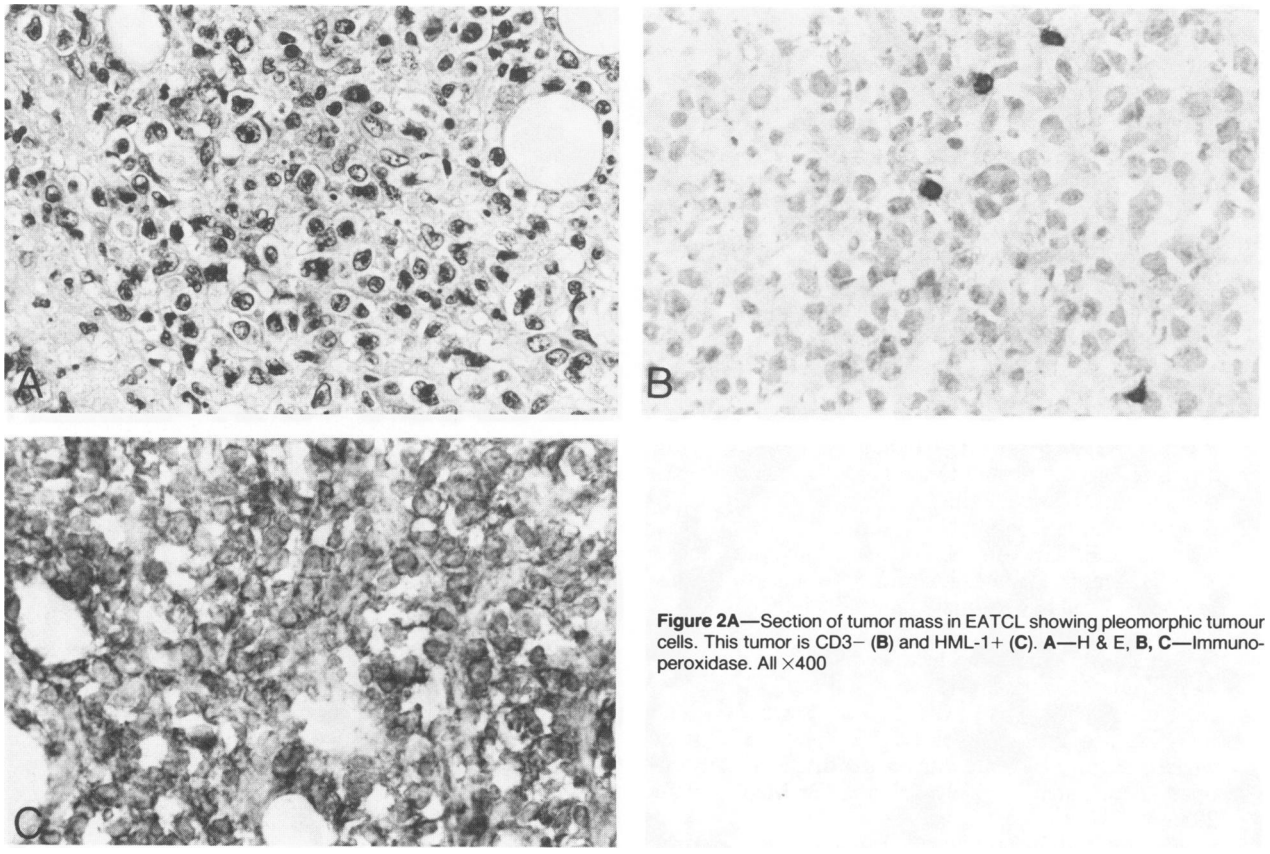

Figure 2A—Section of tumor mass in EATCL showing pleomorphic tumour cells. This tumor is CD3− (**B**) and HML-1+ (**C**). **A**—H & E, **B, C**—Immunoperoxidase. All ×400

expression of the antigen that then persists in daughter cells. That intraepithelial B cells in primary B cell gastric lymphoma are HML-1 negative suggests that if the antigen is induced by a factor derived from epithelium, this factor does not affect all lymphocyte subsets. Alternatively, the antigen recognized by HML-1 may be a marker of mucosal T cell lineage, recognizing cells with an epithelial tropism.

With few exceptions, the phenotype of T cell lymphomas is notoriously variable, even within the same case, and it is therefore difficult, if not impossible, to relate T cell neoplasms to a specific cell of origin.[16] In contrast, EATCL has a relatively uniform phenotype. All cases examined so far have the phenotype HML1+, CD7+, CD4−, CD8−. The expression of CD3 is variable. One case studied expressed CD3 in one tumor mass but not in another, suggesting that this antigen may be lost from the cells as the tumor progresses.[2]

Intraepithelial T lymphocytes are a phenotypically heterogenous population. Most express CD3 and CD8, and a large proportion do not express CD5.[17,18] This contrasts with T cells in peripheral lymphoid tissues and blood, which are predominantly CD3+, CD4+, CD5+.[19,20] In addition, CD7+, CD3+, CD4−, CD8− cells (6% of the total intraepithelial population) and CD7+, CD3−, CD4−, CD8− cells (13% of the total intraepithelial cell population) have been described recently. The latter population is concentrated in the villus tips.[21] The entire heterogenous population is stained by HML-1.[7] Such a degree of heterogeneity within a single population defined by HML-1 suggests that intraepithelial lymphocytes may differentiate within the epithelium, possibly by the progressive loss of surface markers. Studies of preparations of isolated cells and resin embedded tissue have shown that approximately 20–35% of intraepithelial cells contain lysosomal granules.[7,17,22] The malignant cells in EATCL contain lysosomal granules, demonstrated using electron microscopy, a finding that was responsible partly for identification of these cells as neoplastic macrophages in the early stages of research into this lymphoma.[23] The presence of lysosomal cytoplasmic granules in the tumor cells and the exclusive occurrence of EATCL in the mucosa, the frequent occurrence of intraepithelial tumor cells, and the positivity of the tumor cells with HML-1 suggest that EATCL may be a tumor of intraepithelial lymphocytes, possibly of the granular intraepithelial subpopulation.

Detailed phenotypic studies of non-Hodgkin's lymphomas have added little of clinical relevance to established histologic classifications. In describing B cell lymphomas of mucosa associated lymphoid tissue,

Isaacson and Wright suggested that in this condition it is the functional properties of the neoplastic cells rather than their morphology that are clinically relevant.[24] The cytology of EATCL is highly variable and not distinctive within the spectrum of T cell neoplasm as a whole. Clinically, however, EATCL is a well-defined disease with an unusual behaviour centering on its intestinal origin. The identification of a phenotypic marker for EATCL that has functional connotations is a major step forward in the classification of lymphoma.

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